



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/00		A2	(11) International Publication Number: WO 99/62503 (43) International Publication Date: 9 December 1999 (09.12.99)
(21) International Application Number: PCT/EP99/03625	(22) International Filing Date: 26 May 1999 (26.05.99)	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(30) Priority Data: 98109845.2 29 May 1998 (29.05.98) EP 99105693.8 19 March 1999 (19.03.99) EP			(71) Applicant (for all designated States except US): CNRS (CENTRE NATIONAL DE RECHERCHE SCIENTIFIQUE) FRANCE INNOVATION SCIENTIFIQUE ET TRANSFERT [FR/FR]; 135, boulevard Saint Michel, F-75005 Paris (FR).
(71)(72) Applicant and Inventor: EISENBRAND, Gerhard [DE/DE]; Gustav Kirchhoffstrasse 3, D-69120 Heidelberg (DE).			(74) Agent: MÜLLER-BORÉ & PARTNER; Grafinger Strasse 2, D-81671 München (DE).
			<p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>
(54) Title: USE OF INDIGOID BISINDOLE DERIVATIVES FOR THE MANUFACTURE OF A MEDICAMENT TO INHIBIT CYCLIN DEPENDENT KINASES			
<p>The diagram illustrates the cell cycle phases: G₁, S, G₂, and M. Cyclin B is shown associated with cdc2 kinase in the M phase. Arrows indicate the transition from G₁ to S, S to G₂, and G₂ to M. In the M phase, cyclin B is degraded. An arrow labeled "activation by dephosphorylation on T14 and Y15" points to the cyclin B/cdc2 complex in the G₂ phase, indicating its inactivation during the transition to M phase.</p>			
<p>(57) Abstract</p> <p>The present invention relates to the use of indigoid bisindole derivatives for the manufacture of a medicament for inhibiting cyclin dependent kinases, particularly CDK 1, CDK 2, CDK 4 and CDK 5, more particularly ATP:Proteinphosphotransferase p34^{cdc2} (CDK1).</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		

"Use of indigoid bisindole derivatives for the manufacture of a medicament to inhibit cyclin dependent kinases"

Description

The present invention relates to the use of indigoid bisindole derivatives for the manufacture of a medicament for inhibiting cyclin dependent kinases, particularly CDK 1, CDK2, CDK 4 and CDK 5, more particularly ATP:Proteinphosphotransferase p34^{cdc2} (CDK1).

5

Indigoid bisindoles comprise a spectrum of natural dye stuffs. Many of these can be obtained from plants. Accordingly, indirubine, indigo and isoindigo are natural products which can be obtained from different plants: namely, Baphicacanthus cusia (Acanthaceae), Indigofera suffruticosa (Fabaceae), Isatis indigotica (Brassicaceae) and others. Indican, a glycoside which is found in plants, gives glucose and 3-hydroxyindole due to acidic or enzymatic hydrolysis. 3-Hydroxyindole is converted by air-oxidation into indigo and its isomers. Indigo naturalis (Chinese: quingdai) is the natural blue dye obtained from plant material, e.g. Isatis indigotica (Brassicaceae). Indirubine, an isomer of indigo, can be found in Indigo naturalis in an amount of up to 60% (Falbe J. & Regitz M., Römpf Chemie Lexikon (1992), 9. Aufl., Stuttgart, Georg Thieme Verlag). It occurs also in Isatis tinctoria in an amount of up to 5% which is indigenous to Central Europe (Gelius R., Z. Chem., 20, (1980), 340-341).

20

Indigo naturalis is reported to be used in traditional Chinese medicine as a hemostatic, antipyretic, anti-inflammatory and sedative agent in the treatment of bacterial and viral infections. Antileukemic effects of Indigo naturalis have also been reported, with indirubine being the effective principle (Ji X. et al., Acta Pharm. Sin., 16, (1981), 146-148; Gan W. J. et al., J. Hematol., 6, (1985), 611-613). Furthermore, derivatives of indirubine are known for a long time as dyes of low persistence.

Few, structurally quite different types of p34^{cdc2} inhibitors have been described up to now. They are either of natural origin or derived from natural compounds and show varying degrees of inhibitory activities. Examples are Staurosporine, an alkaloid from Streptomyces sp, Butyrolactone-I from Aspergillus terreus var., 5 Flavopiridol, a novel promising anti-tumour agent derived by partial synthesis from a parent structure found in the indian plant Dysoxylum binectariferum, 9-Hydroxyellipticin from the plants Ochrosia elliptica and Ochrosia acuminata, the purine derivatives olomoucine, roscovitine and isopentenyladenine and some peptides. The mechanism of these compounds is based on competitive inhibition 10 of ATP binding (Meijer L., Trends in Cell Biology, 6, (1996), 393-397).

For determination of the IC 50-values: p34^{cdc2}/cyclin B was purified from M phase starfish (*Marthasterias glacialis*) oocytes by affinity chromatography on p9^{CKShs1}-Sepharose beads, from which it was eluted by free p9^{CKShs1} as described 15 (Meijer et al., Eur. J. Biochem., 243, (1997), 527-536). It was assayed with 1 mg histone H1 (Sigma type III-S)/ml, in the presence of 15 µM [γ -³²P] ATP (3,000 Ci/mmol; 1mCi/ml) in a final volume of 30 µl. After 10 min. incubation at 30°C, 25 µl aliquots of supernatant were spotted onto 2.5 x 3 cm pieces of 20 Whatman P81 phosphocellulose paper, and after 20 sec., the filters were washed five times (for at least 5 min. each time) in a solution of 10 ml phosphoric acid/liter of water. The wet filters were transferred into 6 ml plastic scintillation vials, 5 ml ACS (Amersham) scintillation fluid was added and the 25 radioactivity measured in a Packard scintillation counter. The kinase activity was expressed in pmoles phosphate incorporated into histone H1/10 min incubation or in % of maximal activity.

The strongest inhibitors of p34^{cdc2} are derivatives of staurosporine (IC₅₀ = 0,003-0,03 µM). The selectivity of these inhibitors is, however, rather poor. They also show more or less potent inhibitory activity to quite a wide range of 30 cellular kinases. Table 1 shows the specificity of known chemical inhibitors of cyclin-dependent kinases (IC₅₀-values given in µM) (cf. Meijer L., Trends in Cell Biology, 6, (1996), 393-397).

Tab. 1:

Enzyme	Staurosporine	UCN-01	Butyrolactone-I	Flavopiridol	Olomoucine	Roscovitine	9-Hydroxy-ellipticine
5	CDK1	0.003-0.009	0.031	0.60	0.40	7	0.65 ^a ca. 1
	CDK2	0.007	0.030	1.50	0.40	7	0.70 ND
	CDK4	< 10.000	0.032	no effect	0.40	>1000	>100 ND
	MAPK	0.020	0.910	94	ND	30	30 ND
	PKA	0.008	ND	260	145	>2000	>1000 ND
	PKG	0.009	ND	ND	6	>2000	>1000 ND
	PKC	0.005	0.007	160	ND	>1000	>100 ND
	Tyrosine-kinase (EGF-R)	0.006 0.006	ND (Src)	>590	25	440	70 ND

CDK: cyclin-dependent kinase; EGF-R: epidermal growth factor receptor tyrosine kinase; I-R: insulin receptor-tyrosine kinase; MAPK: mitogene-activated protein kinase; ND: not determined; PKA: cAMP-dependent protein kinase;PKG: cGMP-dependent protein kinase.

^aIC₅₀-value for racemic mixture; IC₅₀ for (R)-roscovitin is 0.45 μM.

20

Cyclins and cyclin dependent kinases (CDK) have an essential role for driving the cell through the cell cycle (cell division cycle, cdc). During the cell division cycle, oscillations in concentrations and activities of cyclins are observed. This applies, e.g. to cyclins D and E in the so-called G1-phase of the cell cycle and to cyclinA (S- and M-Phase) and cyclinB (G2- and M-Phase).

25

The cyclin dependent kinases are activated by association with a member of the cyclin-family. Up to now, eight human CDKs have been described: CDK1 (= p34^{cdc2}), CDK2 to CDK8. CDK-proteins consist of a catalytic subunit and a regulatory subunit, the cyclins (Meijer et al., Eur. J. Biochem., 243, (1997), 527-536). Every step of the cell division cycle is regulated by specific CDK/cyclin complexes which ascertain a strict control. Important checkpoints are at the transition from G1 phase to S phase and from G2 phase to M phase (Pines J.,

Cancer Biology, 6, (1995), 63-72). The p34^{cdc2}/cyclinB complexes are important components at the G₂-M-checkpoint.

Fig.1 shows the points of action of the cyclin CDK complex cdc2/cyclinB in the cell division cycle (M = mitosis, cell division; G = gap; S = synthesis; interphase = G₁+S+G₂).

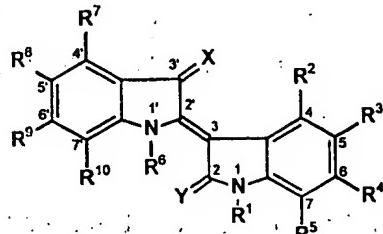
The cdc2/cyclinB complexes are the primary active protein kinases in mitosis. They accumulate in an inactive state in the cytoplasm in interphase cells, and are then rapidly activated by cdc25 phosphatase and translocated into the nucleus at the beginning of mitosis (Pines J., Hunter J., Cell Biol., 115(1), (1991), 1-17). CyclinB is degraded at the metaphase-anaphase transition, inactivating cdc2, which is necessary for exit from mitosis (Glotzer et al., Nature, 349(6305), (1991), 132-138; Murray A.W., Nature, 339(6222), (1989), 280-286; Surana U. et al., Cell, 65(1), (1991), 145-161). Multiple changes of CDK proteins and their regulators are associated with the development of human tumours (Cordon-Cardo C., Am. J. Pathol., 147(3), (1995), 545-560).

Thus, the technical problem underlying the present invention is to provide new inhibitors for cyclin dependent kinases, particularly CDK 1, CDK2, CDK 4 and CDK 5, more particularly ATP:Proteinphosphotransferase p34^{cdc2} (CDK1), which exhibit a high selectivity as well as high efficiency compared to the inhibitors known in the art.

The solution to the above technical problem is achieved by the embodiments characterized in the claims.

In particular, the present invention relates to the use of indigoid bisindole derivatives for the manufacture of a medicament for inhibiting cyclin dependent kinases, particularly CDK 1, CDK2, CDK 4 and CDK 5, more particularly ATP:Proteinphosphotransferase p34^{cdc2} (CDK1), in mammals, preferably in man. Preferably, the indigoid bisindole derivatives are selected from indigo derivatives, isoindigo derivatives or indirubine derivatives.

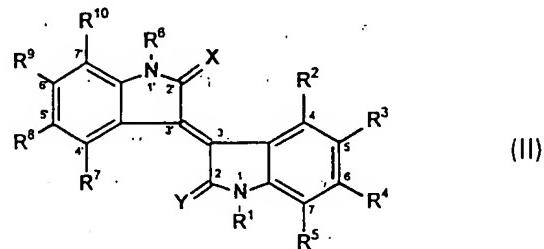
In a preferred embodiment of the present invention, the indirubine derivate is a compound having the general formula (I)



wherein the groups R¹ and R⁶ can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a methylenehydroxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms; a straight-chain or branched-chain alkyloxy group having 1 to 18 carbon atoms; a straight-chain or branched-chain methylenealkoxy group having 1 to 18 carbon atoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a substituted or unsubstituted aralkyl group which can comprise one or more heteroatoms; a substituted or unsubstituted aryloxy group which can comprise one or more heteroatoms; a mono-, di- or trialkylsilyl group having 1 to 6 carbon atoms independently of each other in each instance in the straight-chain or branched-chain alkyl group; a mono-, di- or triarylsilyl group with substituted or unsubstituted aryl groups independently of each other in each instance; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R¹¹ and R¹² can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can

comprise one or more heteroatoms; or an acyl group; a methylene-amino group -CH₂-NR¹¹R¹², wherein R¹¹ and R¹² have the above definitions; a benzyl group, wherein the benzene nucleus can comprise one or more heteroatoms; a methylenecycloalkyl group having 3-to 7 carbon atoms which can comprise one or more heteroatoms; a physiological amino acid residue bound to the nitrogen as an amide; an O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides or disaccharides; or a methylenesulfonate group; R², R³, R⁴, R⁵, R⁷, R⁸, R⁹ and R¹⁰ can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a nitroso group; a nitro group; an alkoxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a substituted or unsubstituted aralkyl group which can comprise one or more heteroatoms; a substituted or unsubstituted aryloxy group which can comprise one or more heteroatoms; a substituted or unsubstituted methylenaryloxy group which can comprise one or more heteroatoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a methylenecycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R¹¹ and R¹² can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group, or wherein the nitrogen atom is part of a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatom(s); a -CONR¹¹R¹² group, wherein R¹¹ and R¹² have the above definitions; a hydroxylamino group; a phosphate group; a phosphonate group; a sulfate group; a sulfonate group; a sulfonamide group; a

- SO₂NR¹¹R¹² group, wherein R¹¹ and R¹² have the above definitions; an azo group -N=N-R¹³, in which R¹³ represents an aromatic system which can be substituted by one or more carboxyl groups, phosphoryl groups or sulfonate groups; or a O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides or disaccharides; or R¹ and R⁵, and R⁶ and R¹⁰, respectively, form independently from each other a ring together having 1 to 4, optionally substituted, CH₂ groups; and X and Y can be the same or different and represent an oxygen atom; a sulfur atom; a selenium atom; a tellurium atom; a NR¹⁴ group in which the group R¹⁴ represents a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can be substituted by one or more carboxyl groups, phosphoryl groups or sulfonate groups; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, an aralkyl group, or a sulfonate group; or a NOR¹⁴ group, wherein the group R¹⁴ has the above definitions.
- With respect to the benzene nuclei constituting the indirubine derivates of the the above general formula (I) one or more ring atoms can be replaced by nitrogen atoms. Further, one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, can be condensed to the indirubine system. Furthermore, the indirubine derivatives having the above general formula (I) can also be bound to a polyethyleneglycolester or a polyethyleneglycoether by ester bondings or ether bondings, respectively.
- In another embodiment of the present invention, the isoindigo derivate is a compound having the general formula (II)

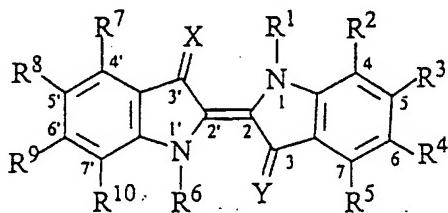


wherein R¹ to R¹⁴ and X and Y have the above definitions.

- 5 With respect to the benzene nuclei constituting the isoindigo derivates of the above general formula (II) one or more ring atoms can be replaced by nitrogen atoms. Further, one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, can be condensed to the isoindigo system. Furthermore, the isoindigo derivatives having the above general formula (II) can be bound to a polyethyleneglycolester or a polyethyleneglycoether by ester bondings or ether bondings, respectively.
- 10

In a further embodiment of the present invention, the indigo derivate is a compound having the general formula (III)

15



20

wherein R¹ to R¹⁴ and X and Y have the above definitions.

- 25 With respect to the benzene nuclei constituting the indigo derivates of the above general formula (III) one or more ring atoms can be replaced by nitrogen atoms. Further, one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, can be condensed to the indigo system. Furthermore, the indigo derivatives having the above general formula (III) can be bound to a polyethyleneglycolester or a polyethyleneglycoether by ester bondings or ether bondings, respectively.
- 30

The above indigoid bisindole derivatives having the general formulas (I), (II) or

(III) can also be in the form of their physiologically acceptable salts.

In the search for new selective inhibitors of cellular signalling pathways, it has surprisingly been found that indigoid bisindole derivatives are highly selective 5 inhibitors of the enzyme complex p34^{cdc2}/cyclinB. An inhibition of p34^{cdc2}-kinase by indigoid bisindole derivatives is not described in the prior art. Surprisingly, it has turned out that indigoid bisindole derivatives are both highly selective and highly effective inhibitors of cdc2-kinase and other cyclin dependent kinases 10 (CDK's), showing IC₅₀-values with the isolated enzyme down to submicromolar range. The inhibition of CDK-activities also is observed in cell culture, using human tumor cell lines. As an example, indirubin-3'-monoxime was found to inhibit histone H1 phosphorylation as a measure for CDK1/cyclin B activity after 24 h incubation of MCF-7 mammary carcinoma cells. Moreover, the content of 15 cyclin B complex was significantly reduced. Cells arrested by serum deprivation after treatment for 24 h with indirubin-3'-monoxime in serum containing medium exhibited an arrest in G1-phase of the cell cycle at low micromolar concentrations of the substance. In concentrations ≥ 5 μM, an additional arrest at G2/M-phase of the cell cycle became apparent. Cells arrested in G2/M by Nocodazole treatment after release of the block exhibited a significant increase 20 of G2/M arrested cells under treatment with ≥ 5 μM indirubin-3'-monoxime, resulting in a massive accumulation of cells in G2/M. Concomitantly with the observed intracellular effects, growth inhibition was induced in the same concentration range, resulting in an IC₅₀-value of 3.3 ± 0.7 μM after 3 days 25 incubation (IC₅₀ = concentration that induces 50% growth inhibition as compared to vehicle control). In addition, induction of apoptotic cell death was observed.

Based on the above mentioned selective inhibitory potency down to the nanomolar range, indirubine derivatives can be used for analytical biochemistry, 30 especially for the study of cell cycle effects. Furthermore, these compounds can be used for the treatment of diseases in patients, which are connected to the loss of proliferation control without any restriction to these potential areas of application. These include cancers, psoriasis, cardiovascular diseases

(stenosis, restenosis) (Brooks et al., J. Biol. Chem., 272, (1997), 29207-2921-1), infectious diseases (unicellular parasites (Trypanosoma, Toxoplasma, Plasmodium, etc.), fungi, etc.), nephrology (glomerulonephritis: Pippin et al., J. Clin. Invest., 100, (1997), 2512-2520), neurodegenerative disorders such as 5 Alzheimer disease (Imahori, K., Uchida T., J. Biochem., 121, (1997), 179-188), viral infections such as cytomegalovirus (Bresnahan W. A. et al., Virology 231, (1997), 239-247) and HIV (Mancebo H.S.Y. et al., Genes & Dev., 11, (1997), 2633-2644).

10 The present invention is explained further by the following examples:

Example 1: Synthesis of indirubine

15 To a solution of 0.42 g (2.4 mmol) of indoxyl acetate in 20 ml methanol under argon 0.35 g (2.4 mmol) of isatin and 0.55 g (5.2 mmol) of sodium carbonate are added. The mixture is stirred for 30 min at ambient temperature. After 24 h standing at ambient temperature, the reaction mixture is filtered off. The precipitate is washed with little methanol and water until the filtrate shows a neutral pH. Residual water is removed by storage in an evacuated exsiccator 20 over potassium hydroxide. Recrystallisation from ethanol or pyridine gives deep purple crystals (Russell G.A., Kaupp G. (1969), J. Am. Chem. Soc., 91, 3851-9, modified).

Yield: 0.51 g (81%), fine, deep-purple needles, Fp: 341-343 °C

25 CHN-analysis: ($C_{16}H_{10}N_2O_2$); MW: 262,26 g/mol; calc.: 73.3% C, 3.8% H, 10.7% N; found: 73.2% C, 4.0% H, 10.6% N

mass spectrum: m/z = 262: (M^+ , 100%), 234: (43%), 205 (25%), 158 (3%), 131 (4%), 103 (7%), 76 (3%)

1H -NMR and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

IR-spectrum: 3340 cm^{-1} : ν (N-H), 1710 cm^{-1} : ν (3'-C=O), 1650 cm^{-1} : ν (2-C=O), 1590 cm^{-1} : ν (C=C, aryl), 1450 cm^{-1} : ν (C=C, aryl), 745 cm^{-1} : ν (aryl with four neighbouring H-atoms).

UV/Vis-spectrum (DMSO): 290 nm, 363 nm, 383 nm (shoulder), 551nm

Essentially the same synthetic procedere was applied for the following Examples 2 to 9 and 11 and 12:

Example 2: 5-Iodoindirubine

5 Yield: 80%, fine, deep-purple needles, Fp: 334-335 °C (decomposition);
CHN-analysis ($C_{16}H_9IN_2O_2$); MG = 388.16 g/mol; calc.: 49.5% C, 2.3% H,
7.2% N; found.: 49.7% C, 2.5% H, 7.1% N;
Mass spectrum: 388 (M^+ , 100%), 360 (3%), 269 (9%), 261 (6%), 233
(16%), 205 (16%), 128 (1%);
10 1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.
UV/Vis-spectrum (DMSO): 370 nm, 386 nm (shoulder), 555 nm.

Example 3: 5-Bromoindirubine

Yield: 70%, fine, deep-purple needles;
15 CHN-analysis ($C_{16}H_9BrN_2O_2$); MG = 341.16 g/mol, calc.: 56.3% C, 2.7% H,
8.2% N; found: 56.4% C, 2.7% H, 8.2% N;
Mass spectrum: 342(M^+ , 100%), 340 (M^+ , 99%), 314 (18%), 262 (64%),
233 (34%), 205 (81%), 177 (10%);
20 1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

Example 4: 5-Chloroindirubine

Yield: 95%, fine, deep-purple needles;
CHN-analysis ($C_{16}H_9ClN_2O_2$); MG = 296.70 g/mol; calc.: 49.5% C, 2.3% H,
7.2% N; found: 49.7% C, 2.5% H, 7.1% N;
25 Mass spectrum: m/z = 296 (M^+ , 100%), 268 (39%), 239 (8%), 233 (35%),
205 (50%), 177 (7%), 153 (6%), 137 (7%), 77 (7%), 120 (4%), 102 (6%),
77 (7%).
30 1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

Example 5: 5-Fluoroindirubine

Yield: 92%, fine, deep-purple needles;
CHN-analysis ($C_{16}H_9FN_2O_2$), MG = 280.25 g/mol, calc.: 68.6% C, 3.2% H,
9.9% N; found: 68.0% C, 3.2% H, 9.9% N;

Mass spectrum: m/z = 281 ($M^+ + H^+$, 19%), 280 (M^+ , 100%), 252 (73%), 223 (32%), 176 (6%), 140 (7%), 121 (13%), 94 (4%), 76 (12%), 77 (7%), 57 (4%), 44 (15%).

1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

5

Example 6: 5-Methylindirubine:

Yield: 92%, fine, deep-purple needles;

CHN-analysis ($C_{17}H_{12}N_2O_2$), MG = 276.28 g/mol, calc.: 73.9% C, 4.4% H, 10.1% N; found: 73.8% C, 4.3% H, 10.2% N;

10

Mass spectrum: m/z = 276 (M^+ , 100%), 261 (10%), 248 (47%), 247 (53%), 220 (6%), 219 (18%), 205 (7%), 171 (4%), 165 (10%), 138 (4%), 133 (15%), 104 (7%), 77 (7%);

1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

15

Example 7: 5-Nitroindirubine

Yield: 88%, fine, deep-purple needles;

CHN-analysis ($C_{16}H_9N_3O_4$), MG = 307.26 g/mol; calc.: 62.5% C, 3.0% H, 13.7% N; found: 62.4% C, 3.0% H, 13.3% N;

20

Mass spectrum: m/z = 307 (M^+ , 5%), 276 (10%), 262 (100%), 234 (23%), 205 (22%), 158 (6%), 131 (10), 104 (19%), 76 (12%), 50 (6%).

1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

Example 8: 5'-Bromoindirubine

Yield: 92%, fine, deep-purple needles;

25

CHN-analysis ($C_{16}H_9BrN_2O_2$), MG = 341.16 g/mol, calc.: 56.3% C, 2.7% H, 8.2% N; found: 55.7% C, 2.5% H, 8.0% N.

1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

Example 9: 5,5'-Dibromoindirubine

30

Yield: 94%, fine, deep-purple needles;

CHN-analysis ($C_{16}H_8Br_2N_2O_2$), MG = 420.06 g/mol; calc.: 45.7% C, 1.9% H, 6.7% N; found: 45.8% C, 2.0% H, 6.4% N.

1H -NMR-spectrum is in accordance with the proposed structure.

Example 10: Indirubine-3'-oxime

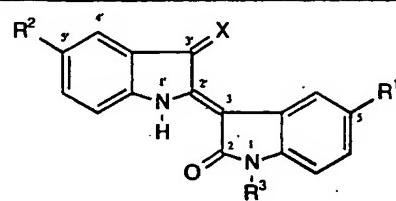
5 Indirubine-3'-oxime was synthesized by reaction of indirubine with hydroxylamine hydrochloride in a pyridine solution (Farbwerke vorm. Meister Lucius & Brüning in Hoechst a.M., Patentschrift des Reichspatentamtes Nr. 283726 (1913)). ^{13}C -NMR-spectroscopy revealed the location of the hydroxyimino residue in 3'-Position ($\delta(\text{C}2) = 171.05 \text{ ppm}$; $\delta(\text{C}3') = 145.42 \text{ ppm}$; DMSO-d₆, RT)

10 Yield: 90 %, red crystals;
CHN-analysis (C₁₆H₁₁N₃O₂), MG = 277.30g/mol; calc.: 69.3% C, 4.0% H, 15.2% N; found: 69.0% C, 4.0% H, 14.9% N;
 ^1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

15 Example 11: Indirubine-5-sulfonic acid
· Yield: 76%, crystalline, deep-purple substance;
Mass spectrum: 388 (M⁺, 100%), 360 (3%), 269 (9%), 261 (6%), 233 (16%), 205 (16%), 128 (1%).
20 ^1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

Table 2 shows the structures of the compounds of Examples 1 to 11.

Tab. 2:



Example	compound	R ¹	R ²	R ³	X
<u>1</u>	Indirubine	H	H	H	O
<u>2</u>	5-Iodoindirubine	I	H	H	O
<u>3</u>	5-Bromoindirubine	Br	H	H	O
<u>4</u>	5-Chloroindirubine	Cl	H	H	O
<u>5</u>	5-Fluoroindirubine	F	H	H	O
<u>6</u>	5-Methylindirubine	CH ₃	H	H	O
<u>7</u>	5-Nitroindirubine	NO ₂	H	H	O
<u>8</u>	5'-Bromoindirubine	H	Br	H	O
<u>9</u>	5,5'-Dibromoindirubine	Br	Br	H	O
<u>10</u>	Indirubine-3'-oxime	H	H	H	NOH
<u>11</u>	Indirubine-5-sulfonic acid	SO ₃ H	H	H	O

Table 3 shows the specificity of the compounds of Examples 1 to 11 (IC₅₀ values given in μ M) to inhibit cdc2 kinase in comparison to other cellular kinases.

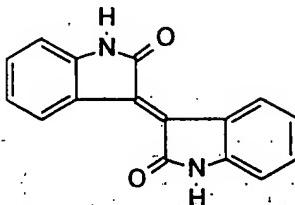
Tab. 3:

Example	cdc2	cdc25	PKA	PKC
<u>1</u>	2	150	no Effect	no Effect
<u>2</u>	0.3	130	no Effect	no Effect
<u>3</u>	0.35	32	ND	ND
<u>4</u>	0.40	55	ND	ND
<u>5</u>	2.0	70	ND	ND
<u>6</u>	0.8	60	ND	ND
<u>7</u>	2.2	60	ND	ND
<u>8</u>	6.5	ND	ND	ND
<u>9</u>	7	ND	ND	ND
<u>10</u>	0.18	ND	ND	ND
<u>11</u>	0.055	110	ND	ND

ND: not detected; PKA: cAMP-dependent protein kinase;
PKC: Ca^{2+} -dependent protein kinase

Example 12: Isoindigo

5



Isoindigo was synthesized by reaction of oxindole with isatin in acetic acid with addition of hydrochloric acid (Wahl A., Bayard P., Comptes Rendues Hébdomadiers des Séances de L'Academie des Sciences, 148, (1909), 716-719).

10 Yield: 84%, crystalline, brown substance;
CHN-analysis ($\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_2$), MG = 262.26 g/mol; calc.: 73.3% C, 3.8% H, 10.7% N; found: 73.0% C, 3.8% H, 10.9% N;
Mass spectrum: m/z = 262 (M^+ , 100%), 234 (85%), 220 (5%), 205 (18%), 190 (4%), 177 (5%), 151 (5%), 132 (17%), 103 (6%), 76 (4%), 32 (26%).
15 $^1\text{H-NMR}$ - and $^{13}\text{C-NMR}$ -spectrum are in accordance with the proposed structure.

Isoindigo shows an IC_{50} -value of $80\mu\text{M}$ for the $\text{p}34^{\text{cdc}2}/\text{cyclinB}$ complex.

20 Table 4 shows the kinase inhibition selectivity of indigo, indirubin, 5-chloroindirubine, indirubine-3'-monoxim and indirubine-5 sulfonic acid. The indicated IC_{50} values were calculated from respective dose response curves and are presented in μM :

25

Tab. 4:

	Enzyme	indigo	indirubine	5-chloro-indirubine	indirubine-3'-monoxime	indirubine-5-sulfonic acid
5	CDK1/ cyclin B	> 1000.000	10.000	0.400	0.180	0.055
	CDK2/ cyclin A	70.000	2.200	0.750	0.440	0.035
10	CDK2/ cyclin E	> 1000.000	7.500	0.550	0.250	0.150
	CDK4/ cyclin D1	> 100.000	12.000	6.500	n.t.	0.300
	CDK5/ p35	> 100.000	5.500	0.800	0.100	0.065

Claims

1. Use of indigoid bisindole derivatives for the manufacture of a medicament for inhibiting cyclin dependent kinases.
 2. Use according to claim 1, wherein the cyclin dependent kinases are selected from CDK 1, CDK 2, CDK 4 or CDK5.
 3. Use according to claim 1 or 2, wherein the indogoid bisindole derivatives are selected from indigo derivatives, isoindigo derivatives or indirubine derivatives.
 4. Use according to claim 3, wherein the indirubine derivate is a compound having the general formula (I)
- (I)

15

20

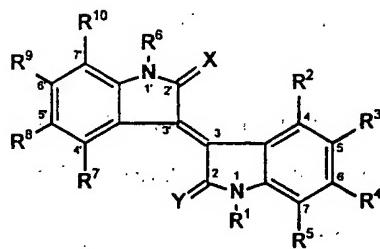
wherein the groups R¹ and R⁶ can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a methylenehydroxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms; a straight-chain or branched-chain alkyloxy group having 1 to 18 carbon atoms; a straight-chain or branched-chain methylenealkoxy group having 1 to 18 carbon atoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a substituted

or unsubstituted aryl group which can comprise one or more heteroatoms; a substituted or unsubstituted aralkyl group which can comprise one or more heteroatoms; a substituted or unsubstituted aryloxy group which can comprise one or more heteroatoms; a mono-, di- or trialkylsilyl group having 1 to 6 carbon atoms independently of each other in each instance in the straight-chain or branched-chain alkyl group; a mono-, di- or triarylsilyl group with substituted or unsubstituted aryl groups independently of each other in each instance; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R¹¹ and R¹² can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; or an acyl group; a methyleneamino group -CH₂-NR¹¹R¹², wherein R¹¹ and R¹² have the above definitions; a benzyl group, wherein the benzene nucleus can comprise one or more heteroatoms; a methylenecycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a physiological amino acid residue bound to the nitrogen as an amide; an O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides or disaccharides; or a methylenesulfonate group; R², R³, R⁴, R⁵, R⁷, R⁸, R⁹ and R¹⁰ can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a nitroso group; a nitro group; an alkoxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a substituted or unsubstituted aralkyl group which can comprise one or more heteroatoms; a substituted or unsubstituted aryloxy

group which can comprise one or more heteroatoms; a substituted or unsubstituted methylenearyloxy group which can comprise one or more heteroatoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a methylenecycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group; wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R¹¹ and R¹² can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group, or wherein the nitrogen atom is part of a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatom(s); a -CONR¹¹R¹² group, wherein R¹¹ and R¹² have the above definitions; a hydroxylamino group; a phosphate group; a phosphonate group; a sulfate group; a sulfonate group; a sulfonamide group; a -SO₂NR¹¹R¹² group, wherein R¹¹ and R¹² have the above definitions; an azo group -N=N-R¹³, in which R¹³ represents an aromatic system which can be substituted by one or more carboxyl groups, phosphoryl groups or sulfonate groups; or a O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides or disaccharides; or R¹ and R⁵, and R⁶ and R¹⁰, respectively, form independently from each other a ring together having 1 to 4, optionally substituted, CH₂ groups; and X and Y can be the same or different and represent an oxygen atom; a sulfur atom; a selenium atom; a tellurium atom; a NR¹⁴ group in which the group R¹⁴ represents a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can be substituted by one or more carboxyl groups, phosphoryl groups or sulfonate groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, an

aralkyl group, or a sulfonate group; or a NOR¹⁴ group, wherein the group R¹⁴ has the above definitions.

5. Use according to claim 4, wherein one or more ring atoms of the benzene nuclei of the compound having the general formula (I) are replaced by nitrogen atoms.
10. Use according to claim 4 or 5, wherein one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, are condensed to the indirubine system.
15. Use according to anyone of claims 4 to 6, wherein the compound having the general formula (I) is bound to a polyethyleneglycolester or a polyethyleneglycoether.
8. Use according to claim 3, wherein the isoindigo derivate is a compound having the general formula (II)

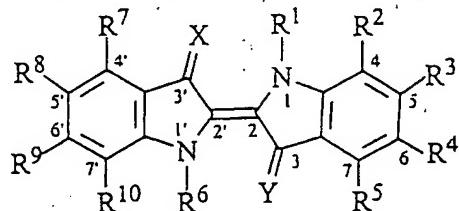


(II)

20. wherein R¹ to R¹⁴ and X and Y have the meanings as defined in claim 4.
25. Use according to claim 8, wherein one or more ring atoms of the benzene nuclei of the compound having the general formula (II) are replaced by nitrogen atoms.

10. Use according to claim 8 or 9, wherein one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, are condensed to the isoindigo system.
- 5 11. Use according to anyone of claims 8 to 10, wherein the compound having the general formula (II) is bound to a polyethyleneglycoester or a polyethyleneglycolether.
- 10 12. Use according to claim 3, wherein the indigo derivate is a compound having the general formula (III)

15



(III)

20

wherein R¹ to R¹⁴ and X and Y have the meanings as defined in claim 4.

25

13. Use according to claim 12, wherein one or more ring atoms of the benzene nuclei of the compound having the general formula (III) are replaced by nitrogen atoms.
14. Use according to claim 12 or 13, wherein one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, are condensed to the indigo system.
- 30 15. Use according to anyone of claims 12 to 14, wherein the compound having the general formula (III) is bound to a polyethyleneglycoester or a polyethyleneglycolether.

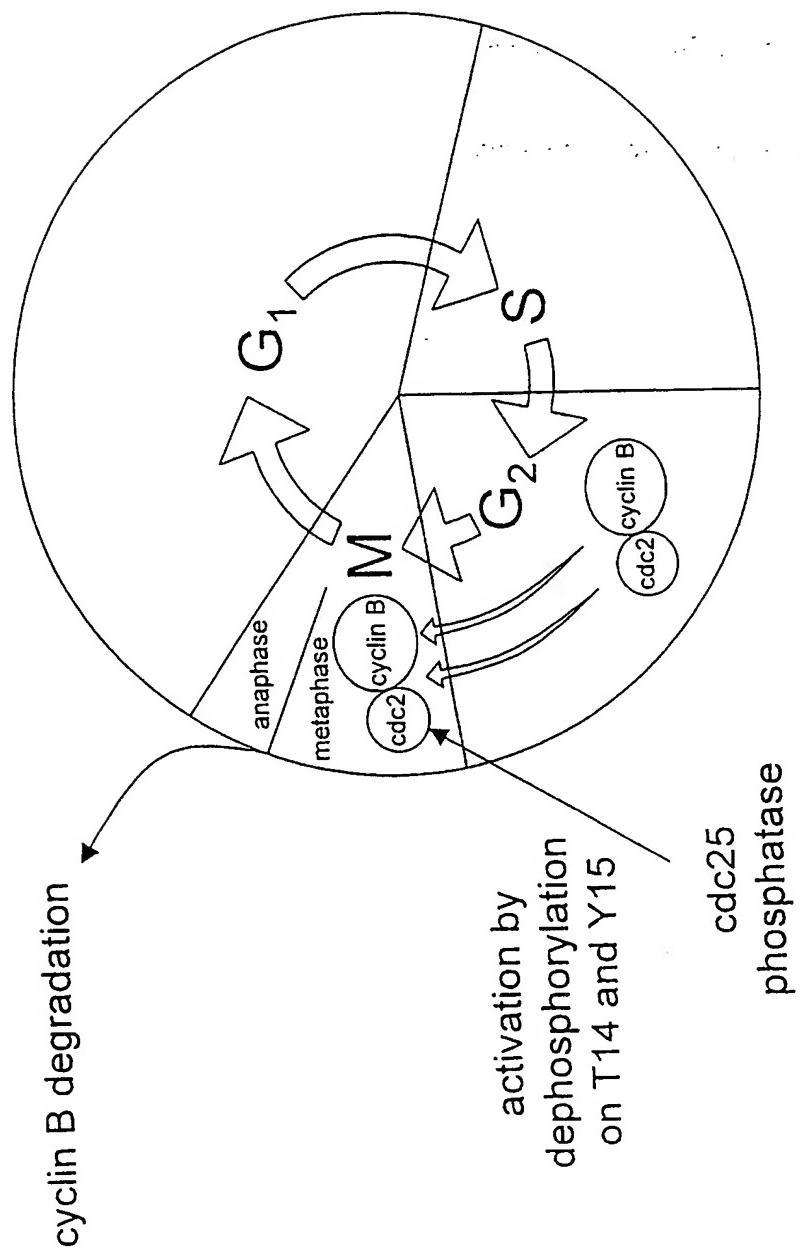
16. Use according to anyone of claims 1 to 15, wherein the indigoid bisindole derivative is in the form of a physiologically acceptable salt.

17. Compound which is 5-fluoroindirubine.

5

18. Compound which is 5-nitroindirubine.

19. Compound which is 5,5'-dibromoindirubine.



SUBSTITUTE SHEET (RULE 26)



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/40, C07D 209/34		A3	(11) International Publication Number: WO 99/62503 (43) International Publication Date: 9 December 1999 (09.12.99)
(21) International Application Number: PCT/EP99/03625 (22) International Filing Date: 26 May 1999 (26.05.99)		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(30) Priority Data: 98109845.2 29 May 1998 (29.05.98) EP 99105693.8 19 March 1999 (19.03.99) EP		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71)(72) Applicant and Inventor: EISENBRAND, Gerhard [DE/DE]; Gustav Kirchhoffstrasse 3, D-69120 Heidelberg (DE).		(88) Date of publication of the international search report: 27 January 2000 (27.01.00)	
(74) Agent: MÜLLER-BORÉ & PARTNER; Grafinger Strasse 2, D-81671 München (DE).			
(54) Title: USE OF INDIGOID BISINDOLE DERIVATIVES FOR THE MANUFACTURE OF A MEDICAMENT TO INHIBIT CYCLIN DEPENDENT KINASES			
(57) Abstract			
The present invention relates to the use of indigoid bisindole derivatives for the manufacture of a medicament for inhibiting cyclin dependent kinases, particularly CDK 1, CDK 2, CDK 4 and CDK 5, more particularly ATP:Proteinphosphotransferase p34 ^{cdc2} (CDK1).			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/03625

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/40 C07D209/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	HOESSEL, RALPH ET AL: "Indirubin, the active constituent of a Chinese antileukemia medicine, inhibits cyclin-dependent kinases" NAT. CELL BIOL. (1999), 1(1), 60-67 , XP002122993 the whole document ----	1-4,8, 12,16 -/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

24 November 1999

Date of mailing of the international search report

08/12/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Veronese, A

INTERNATIONAL SEARCH REPORT

Inter	Final Application No
PCT/EP 99/03625	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US JI, XIUJUAN ET AL: "Antineoplastic effect of indirubin derivatives and their structure-activity relations" retrieved from STN Database accession no. 103:98313 XP002123718 abstract & YAOXUE XUEBAO (1985), 20(2), 137-9 , ---	1-4,8, 12,16
Y	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US ANON.: "Phase II clinical trial on meisoindigo in the treatment of chronic myelogenous leukemia" retrieved from STN Database accession no. 127:214760 XP002123719 abstract & ZHONGHUA XUEYEXUE ZAZHI (1997), 18(2), 69-72 , ---	1-4,8, 12,16
Y	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US GU, Y. C. ET AL: "Synthesis of some halogenated indirubin derivatives" retrieved from STN Database accession no. 112:178548 XP002123720 abstract & YAOXUE XUEBAO (1989), 24(8), 629-32 , ---	1-4,8, 12,16
Y	PATENT ABSTRACTS OF JAPAN vol. 010, no. 148 (C-350), 29 May 1986 (1986-05-29) & JP 61 007254 A (ISUKURA SANGYO KK), 13 January 1986 (1986-01-13) abstract ---	1-4,8, 12,16
Y	WO 98 07695 A (HIRTH KLAUS PETER ;SHAWVER LAURA KAY (US); SUGEN INC (US); TANG PE) 26 February 1998 (1998-02-26) claims ---	1-4,8, 12,16
A	HUNTER T ET AL: "CYCLINS AND CANCER II: CYCLIN D AND CDK INHIBITORS COME OF AGE" CELL,US,CELL PRESS, CAMBRIDGE, MA, vol. 79, page 573-582 XP002069793 ISSN: 0092-8674 the whole document ---	1-4,8, 12,16

-/-

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/03625

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ARTUR BURGER ET AL.: "Pharmaceutisches Wörterbuch" 1998 , DE GRUITER , BERLIN XP002123717 page 704-705	1,2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 99/03625

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 5,6,9,10,13,14 and part of 7,11,15,16

Present claims 1-2 relate to the manufacture of a medicament for inhibiting cyclin dependent kinases (CDK). This, however, is not a specified therapeutic application. Implicitly this medicament is intended for the treatment of diseases related to CDK modulation; the diseases for which the medicament is intended are however not defined in the claims. The use of the definition "medicament for inhibiting cyclin dependent kinases" is considered to lead to a lack of clarity within the meaning of article 6 PCT. The lack of clarity is such to render a meaningful complete search impossible. Consequently the search has been restricted to the diseases mentioned in the description at pages 9-10.

Present claims 5,6,9,10,13,14, relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for none of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds

mentioned in the description, in claims 17-19, compounds corresponding to Markush formula I, II and III, closely related compounds, and for the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/03625

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
JP 61007254	A 13-01-1986	NONE		
WO 9807695	A 26-02-1998	AU 4155697 A EP 0929520 A		06-03-1998 21-07-1999